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The prognostic value of serum osteopontin, HIF-1alpha and pO2 measurements in advanced head and neck tumors treated by radiotherapy.

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Background: Several studies have shown that tumor hypoxia adversely influences prognosis after radiation therapy. But there is a need for clinically useful hypoxia specific prognostic assays. The aim of this study was to compare osteopontin (OPN) shown to be expressed in hypoxic cells with hypoxia inducible factor 1α (HIF-1α) and measurement of physiological tumor oxygenation and to evaluate the prognostic value of these assays in advanced head and neck cancer.

Methods: The study included a total of 55 patients with primary head and neck cancer that all received primary radiation therapy. Serum samples were analysed for OPN using an Elisa assay, histological sections were subjected to immunohistochemical analysis for HIF-1αand tumor oxygenation measurements were made with the Eppendorf oxygen electrode. Endpoints were loco-regional tumor control (LC) and disease specific survival (DSS) at 5 years.

Results: There was large inter patient variability in all 3 measures of hypoxia. Serum OPN had a median of 628 ng/ml (range 168-3790). Absence of HIF-1αstaining was found in one third of patients, between 0-50% HIF-1αstaining in another third and >50% HIF-1αin the final third. Measurements of tumour oxygenation showed a median pO_2 of 10 mmHg (range 0-54) and percentage of values ≤ 2.5 mmHg ($HP_{2.5}$) a median of 28% (range 0-95). The correlation between OPN and median tumour pO_2 was statistically significant (p=0.03) whereas OPN and HIF-1α did not correlate (p=0.14). In survival analysis, when grouping patients into 3 of either OPN, HIF-1α or $HP_{2.5}$, patients with the highest levels of OPN and $HP_{2.5}$ had significantly poorer LC probabilities (p=0.01 and p=0.001, respectively) while patients with HIF-1αscores >50% also did poorly in LC though not statistically significant (p= 0.22). Using DSS as the endpoint, patients with high OPN or HIF-1α labelling above 50% had a statistically significant poorer prognosis (p=0.009 and p=0.05, respectively) while there was a trend that patients with high $HP_{2.5}$ did worse (p≈ 0.06).

Conclusions: OPN correlated with pO_2 but not HIF-1α. Patients with low tumor pO_2 had poorer LC at five year whereas high HIF-1α labelling had a negative effect on DSS. However, OPN was prognostic for both LC and DSS.

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Quality assurance report of EORTC 26981/22981 trial on radiotherapy vs radiotherapy and temozolomide for newly diagnosed glioblastoma multiforme: individual case review

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For the EORTC Radiotherapy and Brain Group Purpose: To assess the compliance to protocol guidelines, where conventionally fractionated focal irradiation is used. The analysis focused on dose-volume evaluation and radiotherapy procedures.

Methods: All participating centres (84) were asked to send data on a randomly selected patient. The required data included surgery, pathology and radiology reports, relevant imaging, simulation films, treatment charts, plans and portal films along with a completed short technology questionnaire. Parameters, related to radiotherapy preparation and execution were analyzed.

Results: Up to now, 35 (41%) centres responded. All of the patients are eligible without any discrepancies between actual and reported data on type of surgery and tumour localization. All centres use immobilization devices and 2D or 3D CT based treatment planning. Field shaping is done by customized blocks (24) or MLC (11). All centres use appropriate photon energy (≥ 4 MV). Treatment verification is done by only portal films (27), only EPID (2), portal films &EPID (2) and portal films & TLD (4). The frequency of portal imaging was at least once (20), weekly (11) or unknown (4). The majority of centres delineate PTV and organs at risk. All centres produce dose distribution plots and 29 centres compute DVH. All patients received 60 Gy in 30 fractions as stated in the protocol except for 2, where a field reduction was necessary to comply with the dose-volume constraints of the protocol. Overall treatment time was ≤ 6 weeks in the vast majority (33). All centres specify the dose to the isocenter of the beams. The maximum and minimum doses within the PTV are reported in 28 (80%) centres. Dose homogeneity is according to ICRU 50 for 18 (50%) centres. PTV coverage is ≥95% for 13, 95% for 5 and 90% for 10 patients, respectively. The under-dose is limited to a very small part of the PTV for all cases. The maximal dose to the optic chiasm and brain stem is below the tolerance level in the majority of patients. For 4 cases, the maximal dose exceed slightly tolerance dose and it was unknown in 5.

Conclusion: All responding centres have the technical capabilities to deliver radiation according to the protocol guidelines. The performance of dose planning is at least level 2 according to ICRU 50 in the majority of centres. Overall, the compliance to the protocol requirements is very good to excellent.

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GENEPI - the European normal and tumor tissue bank and database: a new ESTRO activity

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Background: Recent progress in molecular and cell biology shows strong evidence for a genetic basis for radiation responses in normal tissues as well as in tumours. To create an infrastructure for molecular research in irradiated patients, the ESTRO-GENEPI project was initiated. The aim of GENEPI is to create a European tissue bank linked to a detailed outcome-database of a large cohort of patients receiving radiotherapy.

Material & Methods: The structure of the GENEPI project will be presented in detail.

Results: A normal and tumour tissue bank from irradiated patients with H&N, breast, rectal or prostate cancer will be linked to a detailed clinical outcome database. A central database will be established to provide a link to existing decentral databases and tissue banks. This will foster optimal utilisation and access to data and material. Protocols for outcome assessment, tissue handling, and use and access of the infrastructure will be developed. Furthermore, protocols for inclusion of patients by European centers into this project will be set. Ethical and legal aspects within the European context will be evaluated. It is planned to store lymphocytes, skin, mucosa and tumour tissue of cancer patients for at least 20 years and keep it available for future large scale research projects.

Conclusion: The GENEPI project of ESTRO will generate a clinical, biological and biostatistical network that is expected to become an important resource for biomedical research in the field of radiation biology and effects of radiation therapy.

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Biological assessment of mixed beam irradiation of carbon-ion and X-ray

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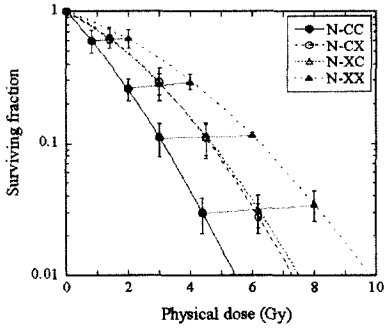
Background: Carbon-ion beam therapy is a promising technology because of its superb biological effects, even for radioresistant tumors, and excellent dose distribution. However, serious adverse effects have also been reported due to the difficulty of avoiding irradiating normal tissues. Moreover, it is an expensive technology, and requires a lot of human resources and time. Mixed beam irradiation with X-ray may provide an answer to these problems. Therefore we researched the biologic effects of mixed beam irradiation of carbon-ion and X-ray at Hyogo Ion Beam Medical Center to assess its possible clinical applications.

Material and Methods: Cultured cells from human salivary gland cancer (HSG cells) were used for all experiments. The following conditioned cells were prepared: cultured under standard condition (Normal), cultured under hypoxic condition for 24 hours before irradiation (Hypoxia), and synchronized in late S-phase of cell cycle by serum starvation technique (Synchronized); Hypoxia and Synchronized are both radioresistant conditions. Cells were irradiated with 320 MeV carbon-ion only (CC), 4 MV X-ray only (XX), or

mixed beam of both (carbon-ion followed by X-ray; CX or X-ray followed by carbon-ion; XC). When utilizing mixed beam, two irradiations were done within 15 minutes. Irradiated doses were determined according to our previous assessment of biologic equivalent doses. Next, 72-hour-interval fractionated irradiation was performed with cells cultured under standard condition (Interval) to observe the difference of sublethal damage repair. Cell survival was assessed with the usual colony formation assay and survival curves were fitted by linear-quadratic model.

Results: In all experiments, the survival curves for cells irradiated with carbon-ion showed the steepest curves with the smallest shoulders, X-ray-irradiated cells showed the gentlest curves with the largest shoulders, and mixed beam irradiation showed intermediate curves. The difference of cell survival in the irradiation sequence of carbon-ion and X-ray (CX or XC) was not significant. In Hypoxia, Synchronized, and Interval conditions, surviving fractions were generally higher than in Normal condition, but not statistically significant. In mathematical analyses, mixed beam irradiation of carbon-ion and X-ray had no synergistic effect, and its cell-killing effect could theoretically be estimated from survival curves of carbon-ion and X-ray by using geometric internal dividing point method. These findings were observed in Hypoxia, Synchronized, and Interval conditions as well as in Normal condition.



Conclusions: The therapeutic effect of mixed beam irradiation of carbon-ion and X-ray is intermediate between carbon-ion only and X-ray only, and can be estimated without any complicated calculations. This provides very important information for the clinical use of mixed beam irradiation.

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Inhibition of angiogenesis and ionizing radiation: treatment-dependent influence on the tumor microenvironment

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Background: The combined treatment approach using inhibitors of angiogenesis (IoA) and ionizing radiation (IR) is a promising strategy against solid tumors. Several preclinical studies demonstrated that IoA enhance radiation-induced tumor growth control but so far the mechanism of combined treatment effect *in vivo* is far from clear. Here we investigate the effect of different treatment modalities on the tumor angiogenic system and on tumor hypoxia with innovative imaging techniques.

Material and methods: *In vivo* growth control experiments (IR (4x3Gy), PTK787 alone and in combination) were performed with tumor allografts derived from the murine c-neu (erbB2) over-expressing breast cancer cell line NF9006. The effect of different treatment modalities on the three-dimensional tumor vessel morphology was assessed by mercox casting followed by electron microscope scanning. Analysis of tumor hypoxia was assessed by 18F-fluoromisonidazole ([18F] FMISO) PET. Expression of distinct angiogenesis and microenvironment parameters was analyzed by immunohistochemistry.

Results: The combined treatment regimen exerted an at least additive growth control effect in NF9006 tumor allografts. Analysis of the different microvessel structures revealed that a distinct angiogenic phenotype resulted in a treatment-dependent way. Whereas in control tumors the morphological pattern of sprouting angiogenesis predominated, treatment with PTK787 (and to a certain extent) with IR alone drastically changed the pattern to intussusceptive microvessel growth. Furthermore combined treatment with PTK787 and IR markedly damaged and shrinked tumor vessels with dramatically reduced microvessel density and total vessel volume. Analysis of tumor hypoxia indicated that treatment with PTK787 alone increased tumor hypoxia to a higher extent than combined treatment or IR alone.

Conclusions: Treatment with PTK787 and/or IR changes the intratumoral angiogenic system as investigated on the morphology and oxygenation level. The treatment-induced angiogenic switch from sprouting to intussusceptive angiogenesis might be part of a treatment-induced tumor environmental stress response.

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Comparison of in vitro growth-inhibitory activity of paclitaxel and docetaxel on squamous cell carcinoma under normoxic and hypoxic conditions during irradiation

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Background: Hypoxia may influence tumor biology and physiology reducing cytotoxic effects of anticancer therapy. Our privious studies showed that docetaxel is more potent to kill hypoxic cancer cells *in vitro*. It is generally believed that both of taxanes, paclitaxel and docetaxel, are promoters of apoptosis in cancer cells. However, the apoptotic mechanisms of paclitaxel and docetaxel in cancer cells under the low concentration of oxygen are still not enough clear.

Materials and methods: Human squamous cell carcinoma cell line, A 431, was treated with paclitaxel, docetaxel and gamma-ray irradiation under normoxic and hypoxic conditions. Growth inhibition and induction of apoptosis were studied by SRB assay, flow cytometric analysis and M30-Apoptosense ELISA. Expression of p53, bax, bcl-2, bcl-XL, HIF-1α was investigated by Western blotting.

Results: Continious paclitaxel and docetaxel exposure over 96 h resulted in a dose-dependend decrease in the survival of A431 tumor cells incubated under normoxia. In the lower concentration range from 0,5 nM to 50 nM docetaxel was 1,3-fold more potent in average than paclitaxel. At concentrations above 500 nM both agents exhibited similar cytotoxic activity. Hypoxic treatment conditions significantly affected paclitaxel cytotoxicity in the lower concentration range. Under hypoxic conditions docetaxel (viability of 31,1% ± 1,3) was 2,0-fold more effective than paclitaxel (63,1% ± 6,4) at concentration 5 nM. Paclitaxel and docetaxel showed a synergistic effect with irradiation under normoxia even at the low concentrations. Hypoxic conditions affected synergism of paclitaxel and irradiation. Docetaxel completely maintained its toxicity despite the changed atmospheric incubation conditions. In the analysis of paclitaxel and docetaxel-induced expression of apoptosis-regulating molecules, the most significant changes were observed for HIF-1α, p53, and bcl-2 family members.

Conclusion: Docetaxel is more potent agent to show cytotoxicity in human squamous cell carcinoma under hypoxia, than paclitaxel. The key elements of the high potency of docetaxel are increased expressions of p53 and apoptosis-regulatory genes.

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An investigation of reoxygenation in high risk prostate cancer following high dose-rate (HDR) brachytherapy

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Background: Reoxygenation of hypoxic tumours is believed to occur during a course of radiotherapy, and is one of the basic principles on which fractionated treatment is based. The primary objective of this study was to directly measure changes in prostate oxygenation following a single 10 Gy fraction of high dose-rate (HDR) brachytherapy in men with prostate cancer.

Materials and Methods: The study was approved by the institutional ethics review board. Eligible patients had high risk localised prostate cancer (stage T3, Gleason 8-10, or PSA >20 ng/ml) with no previous cancer therapy (hormones or radiotherapy). Treatment consisted of two separate HDR brachytherapy treatments of 10 Gy, one week apart prior to external beam radiotherapy. Prostate oxygenation was measured using a 20 cm custom made polarographic needle electrode (Eppendorf), with the patient in the dorsal lithotomy position under spinal anaesthesia. The needle electrode was advanced through the perineum using a brachytherapy template under ultrasound guidance in 0.7 mm pilgrim steps. At least four tracks, one in each quadrant, were made (median of 32 pO readings per track). Median pO, and the hypoxic fraction (HF) considered as the percentage of values < 2.5 mm Hg, were obtained for each quadrant. Clinical, imaging and biopsy data were used to determine if the measurements were being made in